HAEMATOLOGICAL RESPONSES OF NILE TILAPIA (*OREOCHROMIS NILOTICUS* LINNAEUS 1758) TO EXPOSURE TO EFFLUENT FROM PALM OIL MILLS

MULIARI MULIARI[1], YUSRIZAL AKMAL[2,*], IRFANNUR IRFANNUR[2], ILHAM ZULFAHMI[3], ALIM ISNANSETYO[4], INDAH ISTIQOMAH[4], MARIA ULFA[5], and AGUNG S. BATUBARA[6]

[1] Department of Marine Science, Faculty of Agriculture, Universitas Malikussaleh, Aceh Utara, Indonesia
[2] Department of Aquaculture, Faculty of Agriculture, Universitas Almuslim, Bireuen, Indonesia
[3] Department of Fisheries Resources Utilization, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia
[4] Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia
[5] Department of Marine Science, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia
[6] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Indonesia
* Corresponding author: drh.yusrizal.akmal.msi@gmail.com

ABSTRACT

Pollution by palm oil mill effluent (POME) is of great concern in Indonesia. POME pollution of the water can adversely affect aquatic organisms, especially fish. This study aims to analyse the effect of POME on the haematology of tilapia (*Oreochromis niloticus*), including red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and haematocrit. A random experimental design was used (CRD) consisting of four treatments and five replications, including a Control (0% POME): Treatment A (10% of LC_{50}-96 hours: 1.565 mg l^{-1}), Treatment B (15% of LC_{50}-96 hours: 2.347 mg l^{-1}) and Treatment C (20% of LC_{50}-96 hours: 3,130 mg l^{-1}). The analysis of blood of fish exposed to POME for 15, 30 and 45 days revealed significant effects ($p < 0.05$) only on Hb and MCH on day 15 and WBC on day 30. This study indicates that exposure to POME can reduce the WBC value of tilapia recorded on day 30 in treatment C to a level lower than that recorded in other treatments. The Hb value recorded for tilapia exposed to POME on day 15 in treatment C was significantly higher than in the other treatments. Furthermore, POME caused a significant decrease in MCH recorded on day 15, with the lowest value recorded in treatment A. Based on the results of this study, POME can cause a significant decrease in WBC recorded on day 30 and MCH on day 15 and increase in Hb on day 15 in tilapia.

Keywords: fish; haematology; POME; pollutant

Introduction

Indonesia is one of the world's largest palm oil producers (Mukherjee and Sovacool 2014; Tyson et al. 2018) and palm oil mill effluent (POME) is having an adverse effect on the environment, especially the aquatic environment. Rivers are very vulnerable to POME contamination because almost every palm oil industry is located in watersheds (Amalia et al. 2013; Syahza and Asmit 2020). The adverse effects of this waste water can be direct or indirect depending on the volume produced and the level of contamination (Guedenon et al. 2012). POME has a significant negative effect on aquatic biota (Neoh et al. 2013), including phytoplankton (Muliari and Zulfahmi 2016), anaerobic bacteria (Arisht et al. 2020), bivalves (Zieritz et al. 2016) and fish (Hashiguchi et al. 2021).

Comprehensive studies on the toxicity of POME to aquatic organisms, especially fish, are still needed in Indonesia. Territorial waters around Indonesia are very vulnerable to POME contamination (McCarthy and Zen 2010). One of the important and interesting physiological parameters to be studied is fish haematology. There are few studies on the toxicity of POME to fish (Owolabi et al. 2021), particularly Nile tilapia (*Oreochromis niloticus* L.), which is widely distributed in Indonesian waters and is able to adapt to highly polluted environments, including water close to oil palm plantations (Rahim et al. 2013; Sarong et al. 2013; Dekar et al. 2018; Irhami et al. 2018).

Haematological studies have been used as indicators of the health of fish in terms of infection with bacteria and viruses, and effect of pollutants (Svobodova et al. 1991). Furthermore, the research of Sahetapy (2013) indicates there is a reduction in the haematocrit value in the grouper (*Epinephelus fuscoguttatus*) exposed to lead at 6.86 mg l^{-1} for 30 days. In addition, Witeska et al. (2011) report haemolysis, nuclear deformation and chromatin condensation in red blood cells of carp (*Cyprinus carpio*) exposed to cadmium at 0.65 mg l^{-1} for 4 weeks. The results of Jahanbakhshi et al. (2014) reveal that there is an increase in the concentration of neutrophils and decrease in the concentration of lymphocytes in *C. carpio* exposed to crude oil waste. The same is also reported for catfish (*Clarias* sp.) exposed to potassium permanganate and *Hoplias malabaricus* to methylmercury and inorganic lead (Darwish et al. 2001; Ribeiro et al. 2006). Decreased haematocrit values and increased erythropoietic levels also occur in fish after exposure to heavy metals (Witeska 2005). However, only the effect of POME on reproduction (Muliari et al. 2018; Zulfahmi et al. 2018; Muliari et al. 2020a) and ontogenesis (Muliari et al. 2020b) in tilapia is reported and the effect of POME on its haematology has yet to be studied. Therefore, a haematological investi-

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gation is required to determine the effect of POME on the physiology and homeostasis of *O. niloticus*.

Materials and Methods

Research location and time

This research was carried out in 2021, including the sub chronic exposure of fish to POME, which was done at the Aquaculture Laboratory, Almuslim University and the measurement of haematological parameters at the Research Clinical Laboratory, Banda Aceh.

Experimental animals

A total of 300 fish (*O. niloticus*) of a weight range of 28–33 g (30.45 ± 0.34) and length range of 10–13 cm (11.62 ± 1.43) were obtained from the Fish Seed Centre (BBI) Batee Iliek, Bireuen Regency. The acclimatization period lasted for seven days and then healthy fish were selected for the experiments (200 fish). A total 30 l of POME was collected from the Bireuen Regency area, then diluted to 10%, 15% and 20% LC_{50} concentration (15.65 mg l^{-1}) of POME (Zulfahmi et al. 2017). This study was approved by the Animal Research Ethics Committee of the Aquaculture Department, Faculty of Agriculture, Almuslim University, Indonesia (Ethic Code No. 007/Aquaculture-FP.Umuslim/VIII/2021).

Experimental design

A completly randomized design (CRD) was used that consisted of four treatments that were replicated five times. The sub-chronic concentration of POME used in each treatment is based on the LC_{50}-96 hours of POME for *O. niloticus* reported in a previous study, which is 15.65 mg l^{-1} (Zulfahmi et al. 2017). Control Treatment (0% POME), Treatment A (10% of LC_{50}-96 hours: 1.565 mg l^{-1}), Treatment B (15% of LC_{50}-96 hours: 2.347 mg l^{-1}) and Treatment C (20% of LC_{50}-96 hours: 3.130 mg l^{-1}). The fish were kept in aquaria (60 × 40 × 30 cm) containing 43 litres of aerated water. There were ten fish per container (total of 200 fish). During the period they were exposed the pollutant fish were fed commercial feed twice a day.

Blood samples were collected from five fish taken at random from each replication on days 15, 30 and 45, while anesthetized with clove oil to avoid suffering. A 1 ml syringe was used to draw 2–4 ml of blood from the caudal fin, which was stored in an EDTA solution. Blood samples were collected at the Aquaculture Laboratory, Almuslim University and transported to the Research Clinical Laboratory, Banda Aceh, Indonesia for further analysis.

Haematological features of the fish were recorded on days 15, 30 and 45, including numbers of red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and haematocrit. Measurements of haematological parameters were done using a haematology analyser (Mindray BC-30s). MCH was determined by comparing the average amount of haemoglobin in erythrocytes. In addition, measurements of water quality, including dissolved oxygen (DO), temperature and pH, were also recorded.

Statistical analysis

Fish haematological parameters including RBC, WBC, Hb, MCHC, MCH, MCV and haematocrit were analysed statistically (one-way ANOVA) with a 95% confidence level using SPSS software version 22 (IBM SPSS Statistics, IBM, Chicago, USA, Macintosh Version).

Results

Red blood cells (RBC)

When compared to the control group, there was no significant difference in RBC values recorded for the different treatments and durations of exposure. The RBC value of *O. niloticus* exposed to POME recorded on day 15 was higher in each treatment compared to the control, especially treatment C (1.74 ± 0.19 10^6/µl). On day 30 RBC values recorded for each treatment was lower than in the control, with the lowest value recorded for treatment C (1.01 ± 0.32 10^6/µl). A higher RBC value was recorded in each treatment on day 45, but not higher than that for the control (1.55 ± 0.22 10^6/µl) (Fig. 1A).

White blood cells (WBC)

Exposure to POME had a significant effect ($p < 0.05$) on WBC recorded on day 30, but not on days 15 and 45. The lowest WBC values were recorded on day 15 in treatment C (64.10 ± 30.00 10^3/µl) and treatment A (64.32 ± 10.32 10^3 µl),

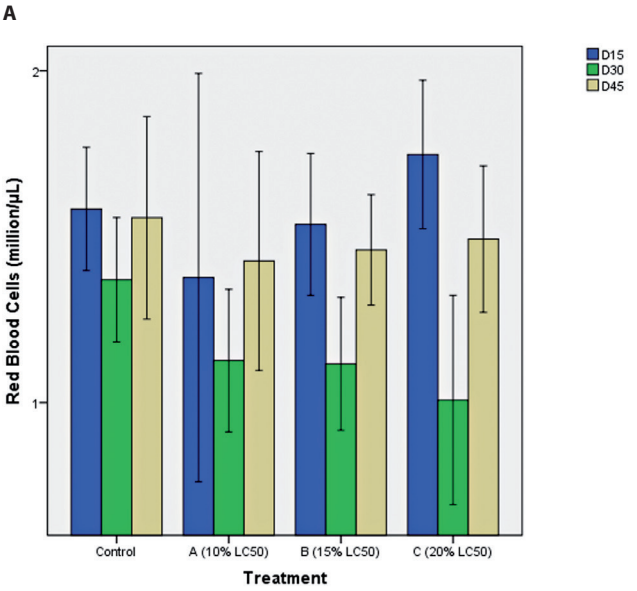
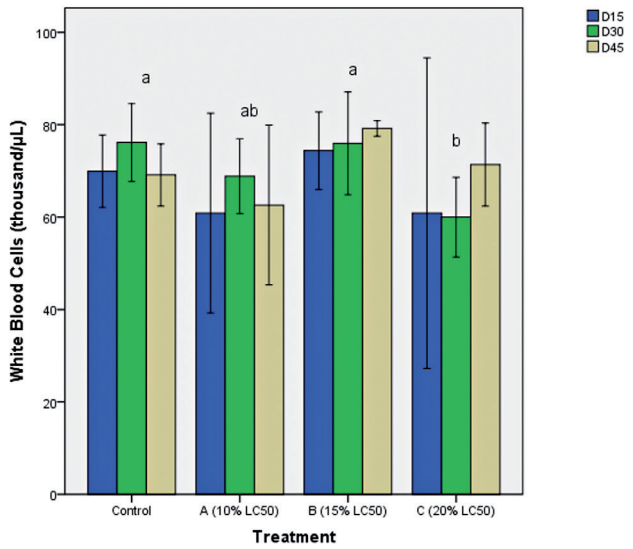
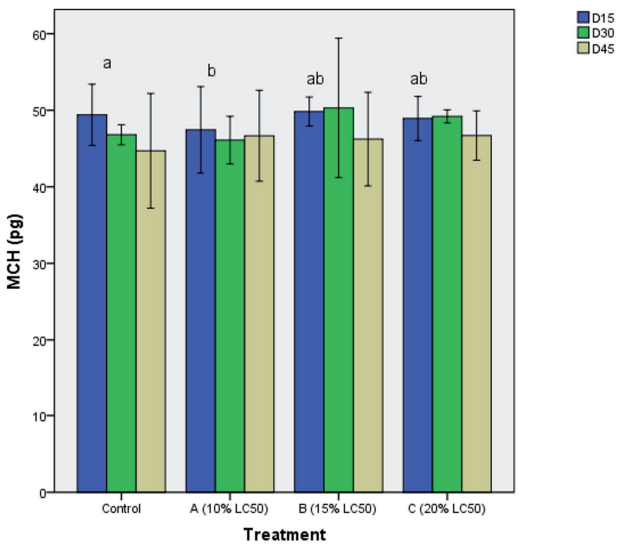


Fig. 1 Graph of (A) RBC, (B) WBC, (C) haemoglobin, (D) MCHC, (E) MCH, (F) MCV and (G) haematocrit values recorded for *Oreochromis niloticus* exposed to POME at different concentrations and periods of time.

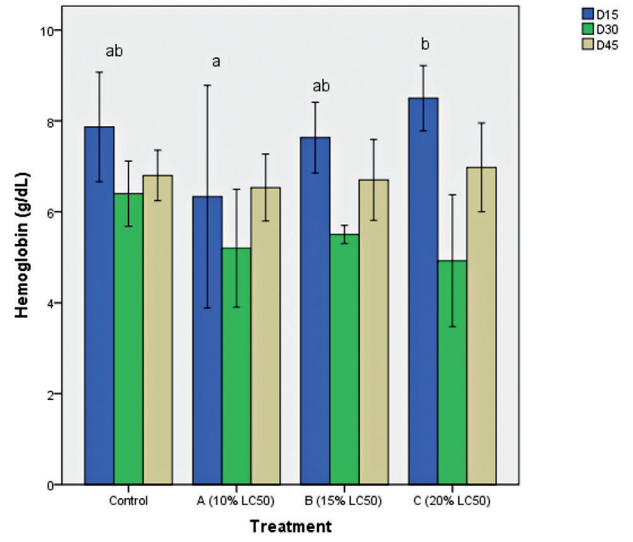
B


White Blood Cells (thousand/µL)
100
80
60
40
20
0
a
ab
a
b
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

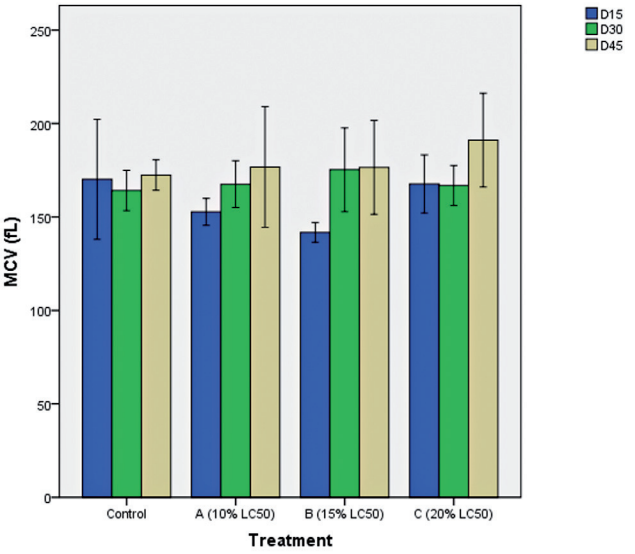
E


MCH (pg)
60
50
40
30
20
10
0
a
b
ab
ab
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

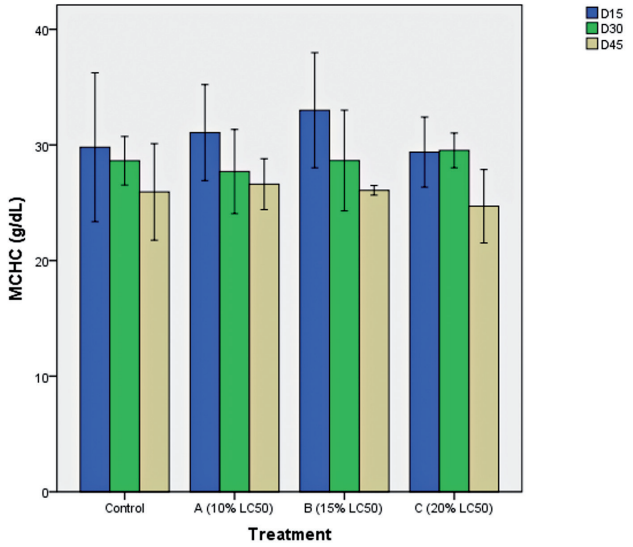
C


Hemoglobin (g/dL)
10
8
6
4
2
0
ab
a
ab
b
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

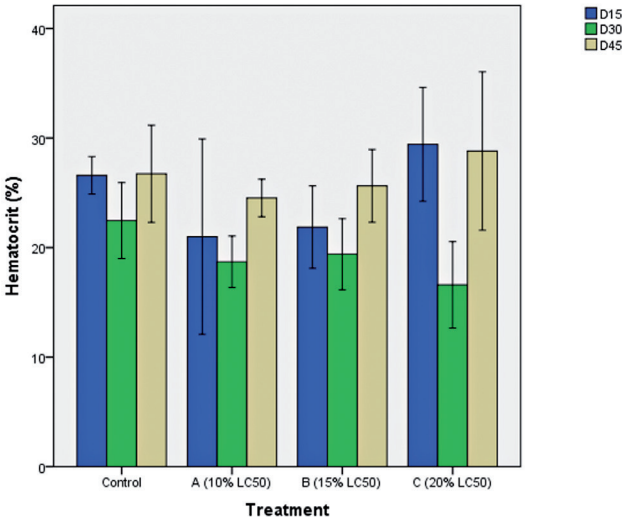
F


MCV (fL)
250
200
150
100
50
0
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

D


MCHC (g/dL)
40
30
20
10
0
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

G


Hematocrit (%)
40
30
20
10
0
Control
A (10% LC50)
B (15% LC50)
C (20% LC50)
Treatment
D15
D30
D45

while the records for the other two treatments were slightly higher. The WBC value for *O. niloticus* in treatment C on day 30 was lower, 59.98 ± 8.61 10^3/µl, while that recorded for the other three treatments tended to be higher, with the highest value recorded for the control (76.13 ± 8.45 10^3/µl). The WBC level recorded for treatment C (72.70 ± 7.98 10^3/µl) on day 45 was higher, while in the other three treatments it was lower, with the lowest value recorded for treatment A (66.84 ± 13.89 10^3/µl) (Fig. 1B).

Haemoglobin (Hb)

Exposure to POME had a significant effect ($p < 0.05$) on Hb recorded on day 15, but not on days 30 and 45. The highest Hb value was recorded on day 15 for treatment C (8.64 ± 0.69 g/dl) and the lowest for treatment A (6.86 ± 1.88 g/dl). The Hb value recorded on day 30 for treatment C was much lower at 4.93 ± 1.45 g/dl as it was in the other three treatments, for which the values were 6.40 ± 0.72 g/dl for the control, 5.50 ± 0.20 g/dl for treatment B and 5.20 ± 1.30 g/dl for treatment A. On day 45, higher values of Hb were record for all treatments, with the highest value recorded for treatment C (7.14 ± 0.92 g/dl) (Fig. 1C).

Mean corpuscular haemoglobin concentration (MCHC)

The MCHC values recorded during the 45 days of this study showed a downward trend in all but treatment A, for which it fluctuated; 29.48 ± 4.58 g/dl on day 15, 27.70 ± 3.64 g/dl on day 30 and 28.20 ± 2.89 g/dl on day 45 (Fig. 1D). The highest MCHC values recorded on days 15, 30 and 45 were recorded for treatment B 33.84 ± 4.71 g/dl, treatment C 29.53 ± 1.50 g/dl and treatment A 28.20 ± 2.89 g/dl.

Mean corpuscular haemoglobin (MCH)

The MCH values recorded differed significantly ($p < 0.05$) on day 15 but not on day 30 and 45 (Fig. 1E). On day 15 the value recorded for the control was higher than that recorded for the other treatments, with values reaching 51.44 ± 4.06 pg, whereas on days 30 and 45 the values recorded for treatment B were 50.30 ± 9.11 pg and 47.92 ± 4.92 pg, respectively.

Mean corpuscular volume (MCV)

The MCV values recorded during the 45 days of the exposure period show an increasing trend (Fig. 1F). The highest values of MCV recorded at each date were for treatment C on days 15 (163.30 ± 16.67 fl) and 45 (189.16 ± 22.12 fl), and treatment B on day 30 (175.27 ± 22.41 fl).

Haematocrit

Haematocrit values recorded for all treatments was higher on day 45, than on days 15 and 30, which fluctuated, with that recorded for each treatment on day 30 low and that on day 45 high (Fig. 1G). The highest values were recorded for treatment C on day 15 (28.70 ± 4.79%) and 45 (29.14 ± 6.30%) and for the control on day 30 (22.47 ± 3.47%).

Water quality

Dissolved oxygen (DO) values differed significantly ($p < 0.05$) depending on the concentration of POME and when sampled. The DO measurements are negatively correlated with the concentration of POME. Furthermore, concentration of POME is also significantly associated ($p < 0.05$) with temperature and pH (Table 2).

Discussion

The decrease in the metabolic rate of fish exposed to pollutants can be expressed in terms of haematological features (Palar 2004). The results of this study revealed that exposure to POME are associated with changes in the number of red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and haematocrit of tilapia. Only Hb and MCH were significantly affected on day 15 of the treatments and WBC on day 30. Different results are reported by Owolabi et al. (2021) for Hybrid Catfish *Heteroclarias,* in which exposure to POME has a significant effect on RBC, MCV and MCHC, and Hb, MCH and WBC.

The results show that RBC recorded for the control was lower than that for the three treatments on day 15, but higher on days 30 and 45. Several types of pollutants are also associated with low RBC values, including copper (Cu), cadmium (Cd), insecticides and nickel (Kang et al. 2005; Al-Akel et al. 2010; Sabilu 2010). The low RBC value of fish exposed to heavy metals is thought to be closely related to the increase in blood viscosity accompanied by damage to RBC (Sabilu 2010). According to Alamanda et al. (2007), a low value of RBC can disrupt the supply of nutrients to cells, tissues and organs, resulting in a reduction in metabolism.

The WBC values of *O. niloticus* exposed to POME were significantly different ($p < 0.05$) on day 30 but not ($p > 0.05$) on days 15 and 45. On the day 30, the WBC value of *O. niloticus* in treatment C 59.98 ± 8.61 10^3/µl, while in the other three treatments it tended to be higher. Similar results are also reported by Owolabi et al. (2021), with 28 days exposure to POME having a significant effect on WBC values in hybrid catfish. Another study on Tor putitora reports significantly higher values of WBC when these fish are exposed to water polluted by industrial waste.

In terms of Hb concentrations, on day 15 of exposure to POME, the highest value (8.64 ± 0.69 g/dl) was recorded for treatment C and the lowest (6.86 ± 1.88 g/dl) for treatment A. On day 30, for treatment C, the Hb levels were low (4.93 ± 1.45 g/dl), as in the other three treat-

Table 1 Haematological values recorded for tilapia exposed to POME at different concentrations and periods of time.

Haematological values	Period of time (days)	Treatments			
		Control (0%)	A (10% LC50)	B (15% LC50)	C (20% LC50)
RBC (10^6/µl)	15	1.46 ± 0.21a	1.58 ± 0.53a	1.51 ± 0.21a	1.74 ± 0.19a
	30	1.37 ± 0.19a	1.13 ± 0.22a	1.12 ± 0.20a	1.01 ± 0.32a
	45	1.55 ± 0.22a	1.48 ± 0.28a	1.33 ± 0.28a	1.53 ± 0.21a
WBC (10^3/µl)	15	68.12 ± 18.98a	64.32 ± 10.32a	73.32 ± 7.20a	64.10 ± 30.00a
	30	76.13 ± 8.45a	68.83 ± 8.11ab	75.97 ± 11.12a	59.98 ± 8.61b
	45	69.64 ± 6.04a	66.84 ± 13.89a	73.70 ± 10.37a	72.70 ± 7.98a
Hb (g/dl)	15	7.54 ± 0.96ab	6.86 ± 1.88a	7.46 ± 0.60ab	8.64 ± 0.69b
	30	6.40 ± 0.72a	5.20 ± 1.30a	5.50 ± 0.20a	4.93 ± 1.45a
	45	6.88 ± 0.64a	6.86 ± 1.01a	6.32 ± 1.26a	7.14 ± 0.92a
MCHC (g/dl)	15	32.30 ± 5.73a	29.48 ± 4.58a	33.84 ± 4.71a	30.62 ± 3.83a
	30	28.63 ± 2.10a	27.70 ± 3.64a	28.65 ± 4.36a	29.53 ± 1.50a
	45	26.00 ± 3.41a	28.20 ± 2.89a	26.90 ± 1.88a	24.92 ± 2.80a
MCH (pg)	15	51.44 ± 4.06a	44.88 ± 5.75b	49.85 ± 4.88ab	49.50 ± 2.80ab
	30	46.80 ± 1.31a	46.10 ± 3.12a	50.30 ± 9.11a	49.20 ± 0.86a
	45	44.88 ± 5.54a	46.96 ± 4.23a	47.92 ± 4.92a	46.70 ± 2.79a
MCV (fl)	15	162.64 ± 25.371a	152.92 ± 8.99a	148.28 ± 10.57a	163.30 ± 16.67a
	30	164.13 ± 10.76a	167.50 ± 12.51a	175.27 ± 22.41a	166.90 ± 10.68a
	45	172.86 ± 7.79a	168.12 ± 26.14a	178.40 ± 19.94a	189.16 ± 22.12a
Hematocrit (%)	15	23.78 ± 4.06a	24.22 ± 8.48a	22.42 ± 3.93a	28.70 ± 4.79a
	30	22.47 ± 3.47a	18.70 ± 2.36a	19.40 ± 3.24a	16.60 ± 3.94a
	45	26.74 ± 3.14a	24.4 ± 1.76a	23.78 ± 5.64a	29.14 ± 6.30a

Different superscripts on the same line indicate a significant difference ($p < 0.05$).

Table 2 Water quality in terms of the concentration of POME and period of time.

Water Quality	Period of time (days)	Treatments			
		Control (0%)	A (10% LC50)	B (15% LC50)	C (20% LC50)
DO	15	7.04 ± 0.90b	6.06 ± 1.38b	4.00 ± 0.64a	3.60 ± 0.63a
	30	6.34 ± 1.05b	6.20 ± 1.43b	3.90 ± 0.76a	3.84 ± 0.60a
	45	4.34 ± 0.23b	4.32 ± 0.43b	4.14 ± 0.80b	2.54 ± 0.81a
Temperature	15	28.20 ± 0.45b	28.00 ± 0.00b	27.5 ± 0.50a	28.20 ± 0.27b
	30	29.00 ± 0.00b	29.00 ± 0.00b	28.60 ± 0.55a	29.00 ± 0.00b
	45	28.00 ± 0.00ab	28.00 ± 0.00ab	28.00 ± 0.00ab	28.00 ± 0.00ab
pH	15	8.00 ± 0.06ab	8.05 ± 0.14ab	7.97 ± 0.13a	8.12 ± 0.03b
	30	7.71 ± 0.04b	7.76 ± 0.15b	7.57 ± 0.06a	7.59 ± 0.08a
	45	7.86 ± 0.08b	7.85 ± 0.15b	7.62 ± 0.05a	7.58 ± 0.07a

Different letters on the same line indicate significant differences ($p < 0.05$).

ments. The Hb values on day 45 in all treatments was high, with the highest value (7.14 ± 0.92 g/dl) recorded for treatment C. The Hb value indicates the ability of fish blood to bind and distribute oxygen (Gomez et al. 2020). According to Bastiawan et al. (2001), low haemoglobin values can result in a low metabolic rate and energy utilization. Exposure to other pollutants (e.g. cadmium chloride, arsenic and lead) also reduces haemoglobin levels in tilapia (*Oreochromis niloticus*), catfish (*Clarias batrachus*) and grouper (*Epinephelus fuscoguttatus*) (Sahetapy 2013; Al-Asgah et al. 2015; Kumar and Banerjee 2016).

On day 15, exposure to POME had a significant ($p < 0.05$) effect on the MCH value of *O. niloticus*, but not ($p > 0.05$) on days 30 and 45. Following exposure

to POME the MCH value recorded for the control was higher than for the other treatments on day 15 (51.44 ± 4.06 pg) but the value recorded for treatment B was very much higher on days 30 and 45, reaching 50.30 ± 9.11 pg and 47.92 ± 4.92 pg, respectively. MCH is the haemoglobin level divided by the number of red blood cells (Stockham and Scott 2008). Polizopoulou (2010) states that high MCV and MCH values can indicate a regenerative anaemia response caused by the haemolysis of red blood cells. Decreases in MCHC values are also reported in various types of fish exposed to arsenic, insecticides, dichlorophenoxy acetic acid, copper (Cu) and cadmium (Cd) (Kang et al. 2005; Kumar and Banerjee 2016).

Over the 45-day exposure period, the MCV value in *O. niloticus* increased (Fig. 1F). Treatment C had the highest MCV value on days 15 (163.30 ± 16.67 fl) and 45 (189.16 ± 22.12 fl) and treatment B on day 30 (175.27 ± 22.41 fl). The MCV value indicates the performance of erythrocyte production during the erythropoiesis process (Burgos-Aceves et al. 2019). Similar results are also reported by Kumar and Banerjee (2016) of an increase in the MCV value of catfish (*Clarias batrachus*) exposed to arsenic. The increase in the MCV value is thought to be due to an increase in the number of RBC per unit volume of plasma (Zandecki et al. 2007). Ikhimioya and Imasuen (2007) also argue that an increase in MCV can occur due to the release of immature RBC into the blood.

Haematocrit values for all treatments fluctuated, with a low value recorded on day 30 and a high value on day 45 (Fig. 1G). The values were highest for treatment C on day 15 (28.70 ± 4.79%) and 45 (29.14 ± 6.30%) and for the control it was at its lowest on day 30 (22.47 ± 3.47%). This value tends to be positively correlated with the number of red blood cells (Boggs et al. 2022). In addition, this value also indicates the level of hunger and gender of fish (Jawad et al. 2004; Rashmeei et al. 2022). Sahetapy (2013) also reports similar results, as the exposure to lead at a concentration of 6.86 ppm for 30 days results in this value decreasing from 24.70% to 9.66% in Grouper (*Epinephelus fuscoguttatus*). Decrease in haematocrit values following exposure to pollutants is reported occurring in several species of fish including tilapia (*Oreochromis niloticus*), milkfish (*Chanos chanos*), piava (*Megaleporinus obtusidens*) and rockfish (*Sebastes schlegeli*) (Kim and Kang 2004; Sabilu 2010; Al-Asgah et al. 2015).

Conclusion

The exposure to POME had no significant effect on the haematology of tilapia (*Oreochromis niloticus*), in terms of the number of red blood cells (RBC), haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and haematocrit ($p > 0.05$),with the exception of Hb and MCH on day 15 and WBC on day 30. This study indicates that exposure to POME resulted in a low value of WBC for tilapia on day 30 in treatment C, which was lower than in the other treatments. Exposure to POME also resulted in the Hb value recorded for tilapia on day 15 in treatment C being significantly higher than in other treatments. Furthermore, exposure to POME was associated with a low value of MCH on day 15, especially in treatment A. Based on the results of this study, exposure of tilapia to POME was associated with low values of WBC on day 30 and MCH values on day 15, and significantly high values of HB on day 15.

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